

Application No.: 09/994,573

Docket No.: 251002009400

**PROPOSED CLAIM AMENDMENTS**

1. (currently amended): A method for producing a soluble protein domain comprising:
  - (a) expressing at least two different nucleotide sequences each encoding a fusion protein comprised of a fragment of a starting protein and a protein exhibiting a function,
  - (b) selecting a fusion protein exhibiting said function from among the proteins synthesized in step (a), as comprising a fragment of said starting protein that is a soluble domain, and
  - (c) synthesizing the soluble domain included in the fusion protein selected in step (b) in a cell-free system.
2. (canceled)
3. (previously presented): The method of claim 1, wherein said protein exhibiting a function in step (a) is selected from the group consisting of an enzyme, a binding protein, a luminescent protein and a fluorescent protein, and functional portions thereof.
4. (previously presented): The method of claim 3, wherein said fluorescent protein is a green fluorescent protein or a variant thereof.
5. (currently amended): The method of claim 1, wherein said selecting in step (b) is performed in cells containing said nucleotide sequences by selecting a clone of said cells which exhibits said function.
6. (previously presented): The method of claim 5, wherein said cells are *Escherichia coli* (*E. coli*).
7. (previously presented): The method of claim 1, wherein the fusion proteins are expressed in a cell-free system, and wherein said selecting in step (b) is performed by measuring the function of the fusion proteins.
- 8-9. (canceled)

sd-225750

6

Application No.: 09/994,573

Docket No.: 251002009400

10. (currently amended): A method for producing a soluble protein domain comprising:
- (a) providing an expression vector which expresses a fusion protein of a first protein with a second protein that is a green fluorescent protein or a variant thereof,
  - (b) partially digesting said expression vector with DNA decomposing enzyme to obtain two or more DNA fragments of said vector containing deletions of the nucleotide sequence encoding the first protein,
  - (c) transforming *E. coli* with each of said DNA fragments prepared in step (b) to obtain two or more transformed *E. coli*,
  - (d) isolating a transformed clone that emits fluorescence among the transformed *E. coli* thus identifying a clone containing DNA that encodes a fusion protein with a soluble protein domain,
  - (e) recovering the DNA from the isolated transformed clone, and
  - (f) synthesizing the soluble protein domain encoded on the recovered DNA in a cell-free system.
11. (previously presented): A method for producing a soluble protein domain comprising:
- (a) selecting a fusion protein that exhibits a function characteristic of a functional protein from a plurality of fusion proteins each composed of a first protein which is said functional protein and a second protein which is a candidate soluble domain, wherein in the selected protein said second protein is a soluble domain, and
  - (b) synthesizing a soluble domain that was included in the fusion protein selected from step (a).
12. (previously presented): The method of claim 11, wherein said second protein is encoded by a DNA fragment resulting from a partially digested DNA.

Application No.: 09/994,573

Docket No.: 251002009400

13. (previously presented): A method for producing a soluble protein domain comprising:

- (a) providing an expression vector comprising a DNA encoding a fusion protein comprised of a first protein and a DNA coding for a second protein which is functional;
- (b) treating said vector with a decomposing enzyme to form two or more digested vectors, each vector comprising a fragment of said DNA encoding the second protein;
- (c) expressing fusion proteins encoded on the digested vectors obtained in step (b);
- (d) selecting the fusion protein exhibiting the function characterizing the functional protein among two or more fusion proteins synthesized in step (c) as comprising a soluble domain of said first protein; and
- (e) synthesizing the soluble domain included in the fusion protein selected in step (d) in a cell-free system.

14. (previously presented): The method of claim 13, wherein the selecting of step (d) is performed by transforming cells with the digested vectors, and selecting a clone which exhibits said function in the obtained transformants.

15. (currently amended): A method to synthesize a soluble domain that is a portion fragment of a starting protein which method comprises synthesizing, in a cell-free system, a protein identified as said soluble domain by:

- (a) preparing a multiplicity of fusion proteins, each said fusion protein comprising a functional portion and a fragment of said starting protein,
- (b) assessing each fusion protein for the function of the functional portion; and
- (c) identifying, as a soluble domain, fragments of said protein which are contained in fusion proteins that exhibit the function of the functional portion.

16. (previously presented): The method of claim 15, wherein said preparing is performed in a cell-free system.

17. (previously presented): The method of claim 15, wherein said preparing is performed intracellularly.

sd-225750

8

Application No.: 09/994,573

Docket No.: 251002009400

18. (previously presented): The method of claim 17, wherein said preparing is performed *in vivo* in *E. coli*.

19. (previously presented): The method of claim 15, wherein the functional portion comprises an enzyme, a binding protein, a luminescent protein or a fluorescent protein or functional portions thereof.

20. (previously presented): The method of claim 19, wherein the fluorescent protein is green fluorescent protein or a variant thereof.

21. (currently amended): A method to produce a soluble domain that is a portion of a starting protein which method comprises

- (a) expressing, in each of at least two *E. coli* colonies, a fusion protein comprising green fluorescent protein (GFP) or a variant thereof fused to a fragment of said starting protein and
- (b) identifying a transformed *E. coli* colony that emits fluorescence, whereby a colony comprising a fusion protein containing a fragment that is a soluble domain is identified, and
- (c) producing the soluble ~~protein~~ domain identified in step (b).

22. (previously presented): The method of claim 21, wherein each said fragment is obtained by a process comprising digesting nucleic acid encoding a fusion protein comprising said GFP or variant and said starting protein with a DNA digesting enzyme.

23. (previously presented): The method of claim 22, wherein said digesting is in only either from the 3' or 5' end of the nucleic acid.